Frontiers in Pharmacology, 2018, vol.9, NAUG

QM/MM description of newly selected catalytic bioscavengers against organophosphorus compounds revealed reactivation stimulus mediated by histidine residue in the acyl-binding loop

Zlobin A., Mokrushina Y., Terekhov S., Zalevsky A., Bobik T., Stepanova A., Aliseychik M., Kartseva O., Panteleev S., Golovin A., Belogurov A., Gabibov A., Smirnov I.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2018 Zlobin, Mokrushina, Terekhov, Zalevsky, Bobik, Stepanova, Aliseychik, Kartseva, Panteleev, Golovin, Belogurov, Gabibov and Smirnov. Butyrylcholinesterase (BChE) is considered as an efficient stoichiometric antidote against organophosphorus (OP) poisons. Recently we utilized combination of calculations and ultrahigh-throughput screening (uHTS) to select BChE variants capable of catalytic destruction of OP pesticide paraoxon. The purpose of this study was to elucidate the molecular mechanism underlying enzymatic hydrolysis of paraoxon by BChE variants using hybrid quantum mechanical/molecular mechanical (QM/MM) calculations. Detailed analysis of accomplished QM/MM runs revealed that histidine residues introduced into the acyl-binding loop are always located in close proximity with aspartate residue at position 70. Histidine residue acts as general base thus leading to attacking water molecule activation and subsequent SN2 inline hydrolysis resulting in BChE reactivation. This combination resembles canonical catalytic triad found in active centers of various proteases. Carboxyl group activates histidine residue by altering its pKa, which in turn promotes the activation of water molecule in terms of its nucleophilicity. Observed re-protonation of catalytic serine residue at position 198 from histidine residue at position 438 recovers initial configuration of the enzyme's active center, facilitating next catalytic cycle. We therefore suggest that utilization of uHTS platform in combination with deciphering of molecular mechanisms by QM/MM calculations may significantly improve our knowledge of enzyme function, propose new strategies for enzyme design and open new horizons in generation of catalytic bioscavengers against OP poisons.

http://dx.doi.org/10.3389/fphar.2018.00834

Keywords

Bioscavenger, Butyrylcholinesterase, Computer design, Organophosphorus compound, Paraoxon, Ultrahigh-throughput screening

References

[1] Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., et al. (2015). GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX 1-2, 19-25. doi: 10.1016/j.softx.2015.06.001

[2] Adamo, C., and Barone, V. (1998). Toward chemical accuracy in the computation of NMR shieldings: the PBE0 model. Chem. Phys. Lett. 298, 113-119. doi: 10.1016/S0009-2614(98)01201-9

[3] Aharoni, A., Gaidukov, L., Yagur, S., Toker, L., Silman, I., and Tawfik, D. S. (2004). Directed evolution of mammalian paraoxonases PON1 and PON3 for bacterial expression and catalytic specialization. Proc. Natl. Acad. Sci. U.S.A. 101, 482-487. doi: 10.1073/pnas.2536901100

[4] Buller, A. R., and Townsend, C. A. (2013). Intrinsic evolutionary constraints on protease structure, enzyme acylation, and the identity of the catalytic triad. Proc. Natl. Acad. Sci. U.S.A. 110, E653-E661. doi: 10.1073/pnas.1221050110

[5] Bussi, G., Donadio, D., and Parrinello, M. (2007). Canonical sampling through velocity rescaling. J. Chem. Phys. 126:014101. doi: 10.1063/1.2408420

[6] Chen, C. Y., Georgiev, I., Anderson, A. C., and Donald, B. R. (2009). Computational structure-based redesign of enzyme activity. Proc. Natl. Acad. Sci. U.S.A. 106, 3764-3769. doi: 10.1073/pnas.0900266106

[7] Dahiyat, B. I., and Mayo, S. L. (1997). De novo protein design: fully automated sequence selection. Science 278, 82-87. doi: 10.1126/science.278.5335.82

[8] Dama, J. F., Rotskoff, G., Parrinello, M., and Voth, G. A. (2014). Transition-tempered metadynamics: robust, convergent metadynamics via on-the-fly transition barrier estimation. J. Chem. Theory Comput. 10, 3626-3633. doi: 10.1021/ct500441q

[9] Ellman, G. L., Courtney, K. D., Andres, V. Jr., and Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95. doi: 10.1016/0006-2952(61)90145-9

[10] Frey, B. J., and Dueck, D. (2007). Clustering by passing messages between data points. Science 315, 972-976. doi: 10.1126/science.1136800

[11] Gaus, M., Cui, Q., and Elstner, M. (2012). DFTB3: extension of the self-consistent-charge density-functional tight-binding method (SCC-DFTB). J. Chem. Theory Comput. 7, 931-948. doi: 10.1021/ct100684s

[12] Gaus, M., Goez, A., and Elstner, M. (2013). Parametrization and benchmark of DFTB3 for organic molecules. J. Chem. Theory Comput. 9, 338-354. doi: 10.1021/ct300849w

[13] Grigoryan, G., Reinke, A. W., and Keating, A. E. (2009). Design of protein-interaction specificity gives selective bZIP-binding peptides. Nature 458, 859-864. doi: 10.1038/nature07885

[14] Grimme, S., Antony, J., Ehrlich, S., and Krieg, H. (2010). A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. J. Chem. Phys. 132:154104. doi: 10.1063/1.3382344

[15] Grimme, S., Ehrlich, S., and Goerigk, L. (2011). Effect of the damping function in dispersion corrected density functional theory. J. Comput. Chem. 32, 1456-1465. doi: 10.1002/jcc.21759

[16] Gruden, M., Andjeklovic, L., Jissy, A. K., Stepanovic, S., Zlatar, M., Cui, Q., et al. (2017). Benchmarking density functional tight binding models for barrier heights and reaction energetics of organic molecules. J. Comput. Chem. 38, 2171-2185. doi: 10.1002/jcc.24866

[17] Hayashi, S., Ueno, H., Shaikh, A. R., Umemura, M., Kamiya, M., Ito, Y., et al. (2012). Molecular mechanism of ATP hydrolysis in F1-ATPase revealed by molecular simulations and single-molecule observations. J. Am. Chem. Soc. 134, 8447-8454. doi: 10.1021/ja211027m

[18] Ilyushin, D. G., Smirnov, I. V., Belogurov, AA Jr, Dyachenko, I. A., Zharmukhamedova, Tlu, Novozhilova, T. I., et al. (2013). Chemical polysialylation of human recombinant butyrylcholinesterase delivers a long-acting bioscavenger for nerve agents in vivo. Proc. Natl. Acad. Sci. U.S.A. 110, 1243-1248. doi: 10.1073/pnas.1211118110

[19] Jbilo, O., L'hermite, Y., Talesa, V., Toutant, J. P., and Chatonnet, A. (1994). Acetylcholinesterase and butyrylcholinesterase expression in adult rabbit tissues and during development. Eur. J. Biochem. 225, 115-124. doi: 10.1111/j.1432-1033.1994.00115.x

[20] Jiang, L., Althoff, E. A., Clemente, F. R., Doyle, L., Rothlisberger, D., Zanghellini, A., et al. (2008). De novo computational design of retro-aldol enzymes. Science 319, 1387-1391. doi: 10.1126/science.1152692

[21] Kitz, R., and Wilson, I. B. (1962). Esters of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase. J. Biol. Chem. 237, 3245-3249

[22] Koga, N., Tatsumi-Koga, R., Liu, G., Xiao, R., Acton, T. B., Montelione, G. T., et al. (2012). Principles for designing ideal protein structures. Nature 491, 222-227. doi: 10.1038/nature11600

[23] Korkegian, A., Black, M. E., Baker, D., and Stoddard, B. L. (2005). Computational thermostabilization of an enzyme. Science 308, 857-860. doi: 10.1126/science.1107387

[24] Kossmann, S., and Neese, F. (2010). Efficient structure optimization with second-order many-body perturbation theory: the RIJCOSX-MP2 method. J. Chem. Theory Comput. 6, 2325-2338. doi: 10.1021/ct100199k

[25] Kubar, T., Welke, K., and Groenhof, G. (2015). New QM/MM implementation of the DFTB3 method in the gromacs package. J. Comput. Chem. 36, 1978-1989. doi: 10.1002/jcc.24029

[26] Kuhlman, B., Dantas, G., Ireton, G. C., Varani, G., Stoddard, B. L., and Baker, D. (2003). Design of a novel globular protein fold with atomic-level accuracy. Science 302, 1364-1368. doi: 10.1126/science.1089427

[27] Kulakova, A., Lushchekina, S., Grigorenko, B., and Nemukhin, A. (2015). Modeling reactivation of the phosphorylated human butyrylcholinesterase by QM(DFTB)/MM calculations. J. Theor. Comput. Chem. 14:1550051. doi: 10.1142/S0219633615500510

[28] Laio, A., and Parrinello, M. (2002). Escaping free-energy minima. Proc. Natl. Acad. Sci. U.S.A. 99, 12562-12566. doi: 10.1073/pnas.202427399

[29] Lindorff-Larsen, K., Piana, S., Palmo, K., Maragakis, P., Klepeis, J. L., Dror, R. O., et al. (2010). Improved side-chain torsion potentials for the Amber ff99SB protein force field. Proteins 78, 1950-1958. doi: 10.1002/prot.22711

[30] Lippow, S. M., Wittrup, K. D., and Tidor, B. (2007). Computational design of antibody-affinity improvement beyond in vivo maturation. Nat. Biotechnol. 25, 1171-1176. doi: 10.1038/nbt1336

[31] Lockridge, O. (2015). Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses. Pharmacol. Ther. 148, 34-46. doi: 10.1016/j.pharmthera.2014.11.011

[32] Lockridge, O., Blong, R. M., Masson, P., Froment, M. T., Millard, C. B., and Broomfield, C. A. (1997). A single amino acid substitution, Gly117His, confers phosphotriesterase (organophosphorus acid anhydride hydrolase) activity on human butyrylcholinesterase. Biochemistry 36, 786-795. doi: 10.1021/bi961412g

[33] Lõoke, M., Kristjuhan, K., and Kristjuhan, A. (2011). Extraction Of genomic DNA from yeasts for PCR-based applications. Biotechniques 50, 325-328. doi: 10.2144/000113672

[34] Luo, S., Zhao, Y., and Truhlar, D. G. (2011). Validation of electronic structure methods for isomerization reactions of large organic molecules. Phys. Chem. Chem. Phys. 13, 13683-13689. doi: 10.1039/c1cp20834a

[35] Lushchekina, S. V., Schopfer, L. M., Grigorenko, B. L., Nemukhin, A. V., Varfolomeev, S. D., Lockridge, O., et al. (2018). Optimization of cholinesterase-based catalytic bioscavengers against organophosphorus agents. Front. Pharmacol 9:211. doi: 10.3389/fphar.2018.00211

[36] Malisi, C., Kohlbacher, O., and Hocker, B. (2009). Automated scaffold selection for enzyme design. Proteins 77, 74-83. doi: 10.1002/prot.22418

[37] Mesulam, M. M., Guillozet, A., Shaw, P., Levey, A., Duysen, E. G., and Lockridge, O. (2002). Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. Neuroscience 110, 627-639. doi: 10.1016/S0306-4522(01)00613-3

[38] Michaud-Agrawal, N., Denning, E. J., Woolf, T. B., and Beckstein, O. (2011). MDAnalysis: a toolkit for the analysis of molecular dynamics simulations. J. Comput. Chem. 32, 2319-2327. doi: 10.1002/jcc.21787

[39] Nachon, F., Asojo, O. A., Borgstahl, G. E., Masson, P., and Lockridge, O. (2005). Role of water in aging of human butyrylcholinesterase inhibited by echothiophate: the crystal structure suggests two alternative mechanisms of aging. Biochemistry 44, 1154-1162. doi: 10.1021/bi048238d

[40] Osipovitch, D. C., Parker, A. S., Makokha, C. D., Desrosiers, J., Kett, W. C., Moise, L., et al. (2012). Design and analysis of immune-evading enzymes for ADEPT therapy. Protein Eng. Des. Sel. 25, 613-623. doi: 10.1093/protein/gzs044

[41] Perdew, J. P., Burke, K., and Ernzerhof, M. (1996). Generalized gradient approximation made simple. Phys. Rev. Lett. 77, 3865-3868. doi: 10.1103/PhysRevLett.77.3865

[42] Reshetnikov, R. V., Stolyarova, A. V., Zalevsky, A. O., Panteleev, D. Y., Pavlova, G. V., Klinov, D. V., et al. (2018). A coarse-grained model for DNA origami. Nucleic Acids Res. 46, 1102-1112. doi: 10.1093/nar/gkx1262

[43] Rothlisberger, D., Khersonsky, O., Wollacott, A. M., Jiang, L., Dechancie, J., Betker, J., et al. (2008). Kemp elimination catalysts by computational enzyme design. Nature 453, 190-195. doi: 10.1038/nature06879

[44] Salvat, R. S., Parker, A. S., Guilliams, A., Choi, Y., Bailey-Kellogg, C., and Griswold, K. E. (2014). Computationally driven deletion of broadly distributed T cell epitopes in a biotherapeutic candidate. Cell. Mol. Life Sci. 71, 4869-4880. doi: 10.1007/s00018-014-1652-x

[45] Siegel, J. B., Zanghellini, A., Lovick, H. M., Kiss, G., Lambert, A. R., St Clair, J. L., et al. (2010). Computational design of an enzyme catalyst for a stereoselective bimolecular Diels-Alder reaction. Science 329, 309-313. doi: 10.1126/science.1190239

[46] Smirnov, I. V., Golovin, A. V., Chatziefthimiou, S. D., Stepanova, A. V., Peng, Y., Zolotareva, O. I., et al. (2016). Robotic QM/MM-driven maturation of antibody combining sites. Sci. Adv. 2, e1501695. doi: 10.1126/sciadv.1501695

[47] Socolich, M., Lockless, S. W., Russ, W. P., Lee, H., Gardner, K. H., and Ranganathan, R. (2005). Evolutionary information for specifying a protein fold. Nature 437, 512-518. doi: 10.1038/nature03991

[48] Stein, A., and Kortemme, T. (2013). Improvements to robotics-inspired conformational sampling in rosetta. PLoS One 8:e63090. doi: 10.1371/journal.pone.0063090

[49] Sun, R., Dama, J. F., Tan, J. S., Rose, J. P., and Voth, G. A. (2016). Transition-tempered metadynamics is a promising tool for studying the permeation of drug-like molecules through membranes. J. Chem. Theory Comput. 12, 5157-5169. doi: 10.1021/acs.jctc.6b00206

[50] Sun, R., Sode, O., Dama, J. F., and Voth, G. A. (2017). Simulating protein mediated hydrolysis of ATP and other nucleoside triphosphates by combining QM/MM molecular dynamics with advances in metadynamics. J. Chem. Theory Comput. 13, 2332-2341. doi: 10.1021/acs.jctc.7b00077

[51] Terekhov, S., Smirnov, I., Bobik, T., Shamborant, O., Zenkova, M., Chernolovskaya, E., et al. (2015). A novel expression cassette delivers efficient production of exclusively tetrameric human butyrylcholinesterase with improved pharmacokinetics for protection against organophosphate poisoning. Biochimie 118, 51-59. doi: 10.1016/j.biochi.2015.07.028

[52] Terekhov, S. S., Smirnov, I. V., Stepanova, A. V., Bobik, T. V., Mokrushina, Y. A., Ponomarenko, N. A., et al. (2017). Microfluidic droplet platform for ultrahigh-throughput single-cell screening of biodiversity. Proc. Natl. Acad. Sci. U.S.A. 114, 2550-2555. doi: 10.1073/pnas.1621226114

[53] Tinberg, C. E., Khare, S. D., Dou, J., Doyle, L., Nelson, J. W., Schena, A., et al. (2013). Computational design of ligand-binding proteins with high affinity and selectivity. Nature 501, 212-216. doi: 10.1038/nature12443

[54] Tribello, G. A., Bonomi, M., Branduardi, D., Camilloni, C., and Bussi, G. (2014). PLUMED 2: new feathers for an old bird. Comput. Phys. Commun. 185, 604-613. doi: 10.1016/j.cpc.2013.09.018

[55] Valiyaveettil, M., Alamneh, Y., Rezk, P., Biggemann, L., Perkins, M. W., Sciuto, A. M., et al. (2011). Protective efficacy of catalytic bioscavenger, paraoxonase 1 against sarin and soman exposure in guinea pigs. Biochem. Pharmacol. 81, 800-809. doi: 10.1016/j.bcp.2010.12.024

[56] Vasilevskaya, T., Khrenova, M. G., Nemukhin, A. V., and Thiel, W. (2016). Reaction mechanism of matrix metalloproteinases with a catalytically active zinc ion studied by the QM(DFTB)/MM simulations. Mendeleev Commun. 26, 209-211. doi: 10.1016/j.mencom.2016.04.010

[57] Vellard, M. (2003). The enzyme as drug: application of enzymes as pharmaceuticals. Curr. Opin. Biotechnol. 14, 444-450. doi: 10.1016/S0958-1669(03)00092-2

[58] Weigend, F., and Ahlrichs, R. (2005). Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: design and assessment of accuracy. Phys. Chem. Chem. Phys. 7, 3297-3305. doi: 10.1039/b508541a

[59] Yang, S., Cohen, C. J., Peng, P. D., Zhao, Y., Cassard, L., Yu, Z., et al. (2008). Development of optimal bicistronic lentiviral vectors facilitates high-level TCR gene expression and robust tumor cell recognition. Gene Ther. 15, 1411-1423. doi: 10.1038/gt.2008.90

[60] Yang, Y., Yu, H., and Cui, Q. (2008). Extensive conformational transitions are required to turn on ATP hydrolysis in myosin. J. Mol. Biol. 381, 1407-1420. doi: 10.1016/j.jmb.2008.06.071

[61] Zanghellini, A., Jiang, L., Wollacott, A. M., Cheng, G., Meiler, J., Althoff, E. A., et al. (2006). New algorithms and an in silico benchmark for computational enzyme design. Protein Sci. 15, 2785-2794. doi: 10.1110/ps.062353106

[62] Zheng, F., Xue, L., Hou, S., Liu, J., Zhan, M., Yang, W., et al. (2014). A highly efficient cocaine-detoxifying enzyme obtained by computational design. Nat. Commun. 5:3457. doi: 10.1038/ncomms4457